Histochemical Demonstration of Adrenergic Fibers in the Smooth Muscle Layer of Media of Arteries Supplying Abdominal Organs

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by

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INTRODUCTION

Our present knowledge of sympathetic innervation and vasomotor activity in the splanchnic vasculature is based predominantly on physiological studies. Tbe splanchnic vascular bed is the largest of the regional circulation, about 30% of the total blood circulation. The change of splanchnic circulation plays a great role in the function of digestive organs. Clinically, intestinal ischemia or arterial occlusive lesions of splanchnic areas have been reported by many surgenons. These lesions are now being repaired with the technique of such revascularizations, as endoarterectomy or graft bypass, but the splanchnic vasculature has often remained resistant to surgeons. Therefore, the principles of revascularization learned in the limbs in our clinic are applicable in the splanchnic vascular areas. Thus, sympathectomy is also indicated for intestinal ischemia or arterial occlusive lesions of splanchnic areas, as well as for ischemic change of arterial occlusion in the limbs. It is hoped that the regional sympathectomy may caused vasodilatation of splanchnic vasculature.

Regarding the sympathetic innervation of arteries suppyling abdominal organs, celiac artery, superior mesenteric artery and so on, many investigators have reported the existence of vascular efferent fibers in the adventitia and in the media of the arteries, but there is much argument about the exact localization and ramification of autonomic efferent fibers into the arteries. A specific histochemical fluorescence method for the detection of catecholamines and 5-HT in tissues has recently developed. Several tissues have been shown to be capable of taking up and bind catecholamines and it is now generally accepted that this occurs predominantly in the adrenergic nerves.

Thus, in the course of our experiments to investigate the mode of distribution of adrenergic nerve fibers in differnt vasculatures in the body, we have been able to find a specific fluorescent material representing catecholamines and adrenergic nerve fibers in the smooth muscle layer of the media in the arteries supplying abdominal organs in animals and human.

A group of vessels, which includes the arteries leaving the abdominal aorta, and all branches of these vessels supplying the viscerae, were studied by out experiments. Our experimental studies revealed that these artieries contained advenergic nerve fiber in the media and adventitia.

METHODS AND MATERIALS

Thirty dogs, weighing 10–20 kg, and five rats and ten guine pig, and also several clinical materials were used. Small tissue specimens of arteries, which included celiac, hepatic, splenic, gastric, gastroepiploic and gastroduodenal arteries, and also superior mesenteric and inferior mesenteric arteries, were biopsied from animals anaesthetized with sodium pentobarbital (25 mg/kg, i.v.).

Immediately after excision, the specimens were freeze-dried, treated with paraformaldehyde, embedded in paraffin, sectioned (5–7.5 μ thick) and mounted in Entellan for fluorescence microscopy, as described elsewhere.

Sections, adjacent to those which were exposed to paraformaldehyde for fluorescence microscopy, were stained with hematoxylin-eosin. To intensify the fluorescence of the artery, dogs were treated with noradrenaline (50 μg/kg, i.v.) at various time intervals before excision of specimen. Some animals were given reserprine (0.3 mg/kg, 3 times, i.p.) to deplete catecholamines and test the specificity of the observed fluorescence.

In 4 dogs, chronic resection of the paravertebral sympathetic trunk at the level of Th 3～L 6, including preaortic ganglia, was performed 10 days previously. To differentiate the catecholamine fluorescence from non-specific autofulorescence, sections were treated with sodium borohydride.

RESULTS

Normal distribution of adrnergic nerves to arteries supplying abdominal organs.

Treatment with formaldehyde gas of freeze-dried sections of the arteries supplying abdominal organs of normal animals produced a strong, specific greenish fluorescence.

The fairly rich localization of the fluorescent meterial to the arteries dominates the fluorescent microscopical picture in arterial wall of blood vessels supplying abdominal organs, which are illustrated in Fig. 1, 2, and 3.

Celiac artery of dog (Fig. 4)

Several bundles of fluorescent adrenergic fibers are present in the media of the celiac artery of dog.

Common hepatic artery and proper hepatic artery of dog (Fig. 5 and 6). Several bundles of fluorescent adrenergic fibers are seen in the media of the common hepatic artery of dog.

Fluorescent adrenergic fibers are observed between the media and the adventitia of the proper hepatic artery of dog.

Gastroepiploic artery, gastric artery and gastroduodenal artery. Several bundles of fluorescent adrenergic fibers are observed in the media of the arteries (Fig. 7).

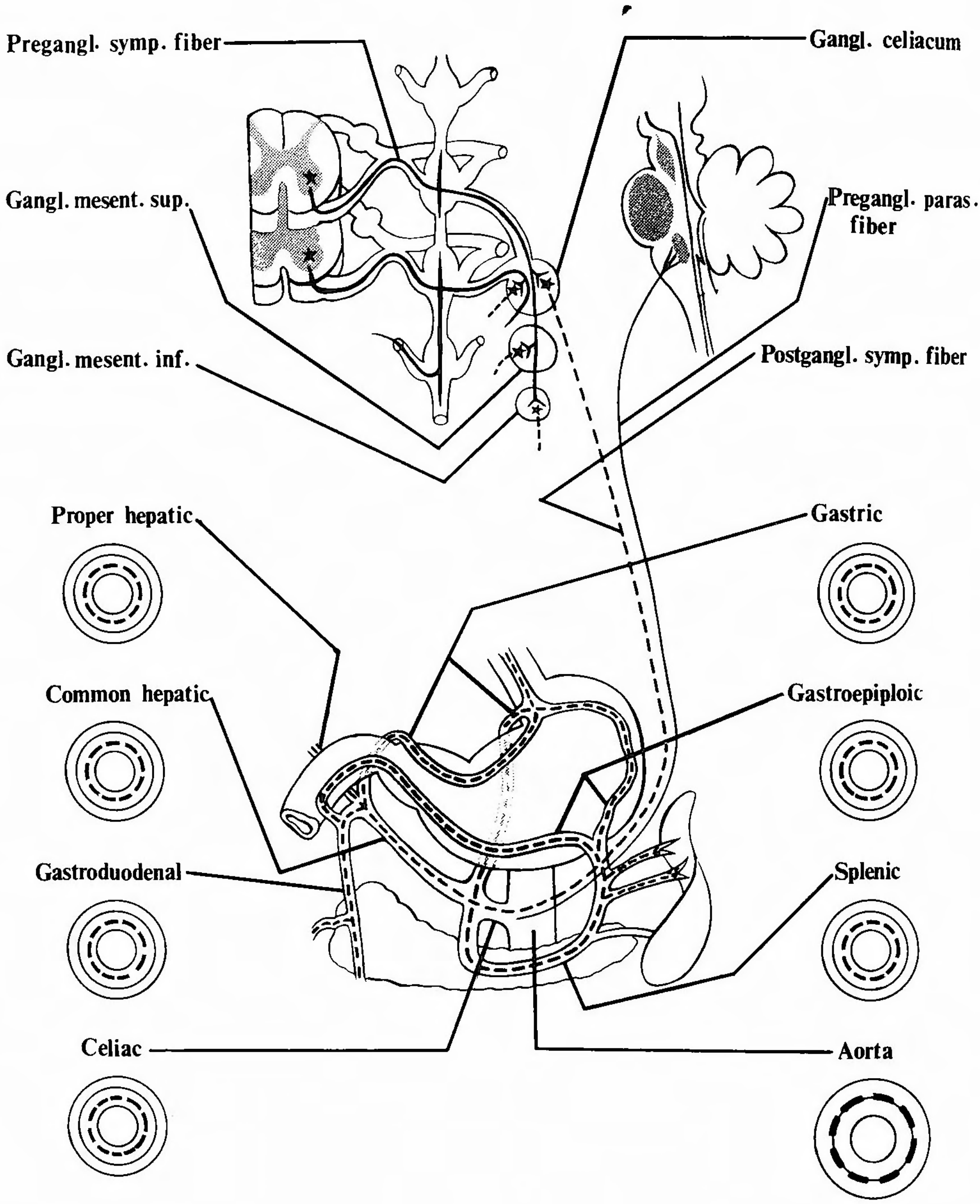

Pregangl. symp. fiber
Gangl. celiacum
Gangl. mesent. sup.
Pregangl. paras. fiber
Gangl. mesent. inf.
Postgangl. symp. fiber
Proper hepatic
Gastric
Common hepatic
Gastroepiploic
Gastroduodenal
Splenic
Celiac
Aorta

Fig. 1

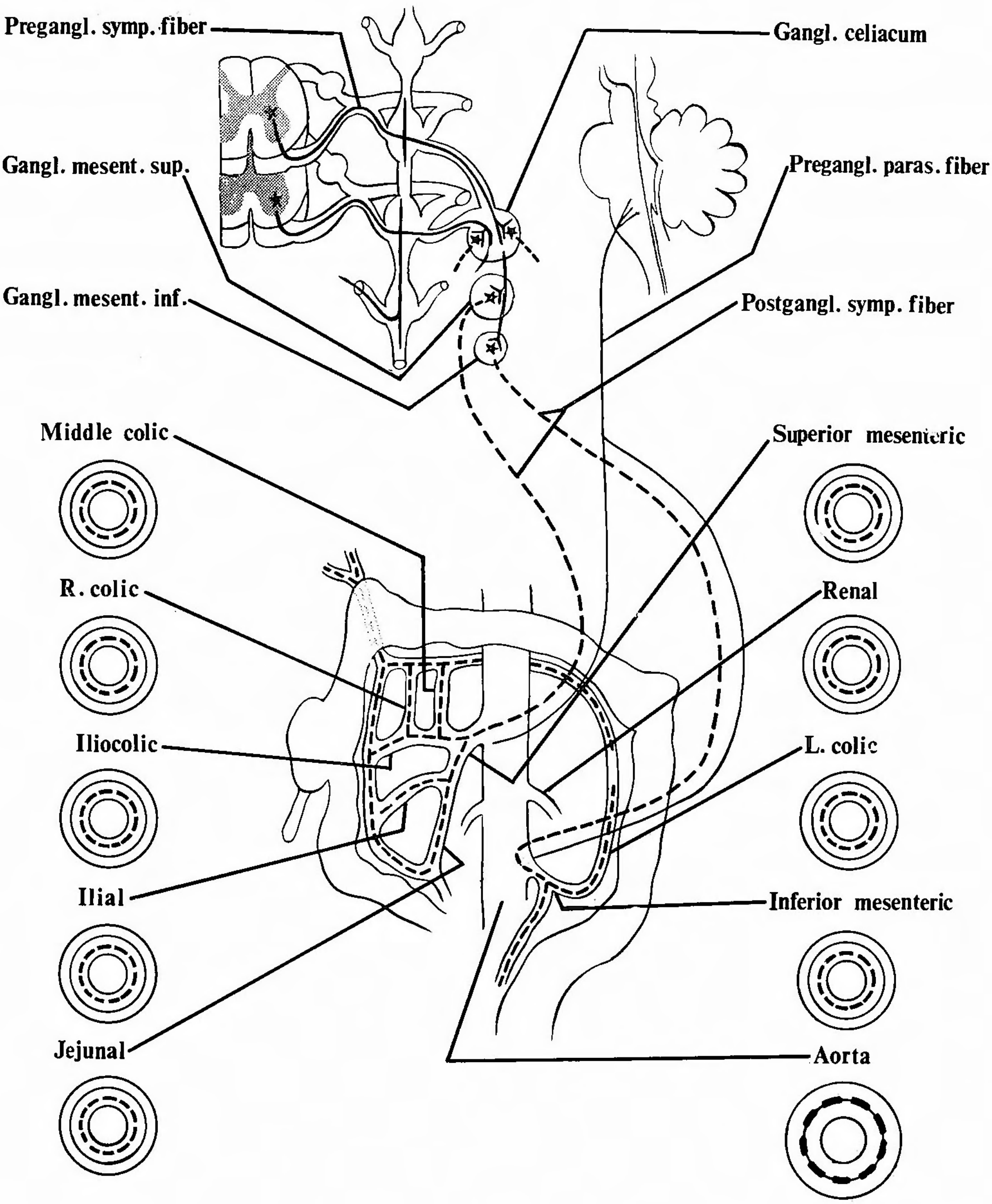

Pregangl. symp. fiber
Gangl. celiacum
Gangl. mesent. sup.
Pregangl. paras. fiber
Gangl. mesent. inf.
Postgangl. symp. fiber
Middle colic
Superior mesenteric
R. colic
Renal
Iliocolic
L. colic
Ilial
Inferior mesenteric
Jejunal
Aorta

Fig. 2

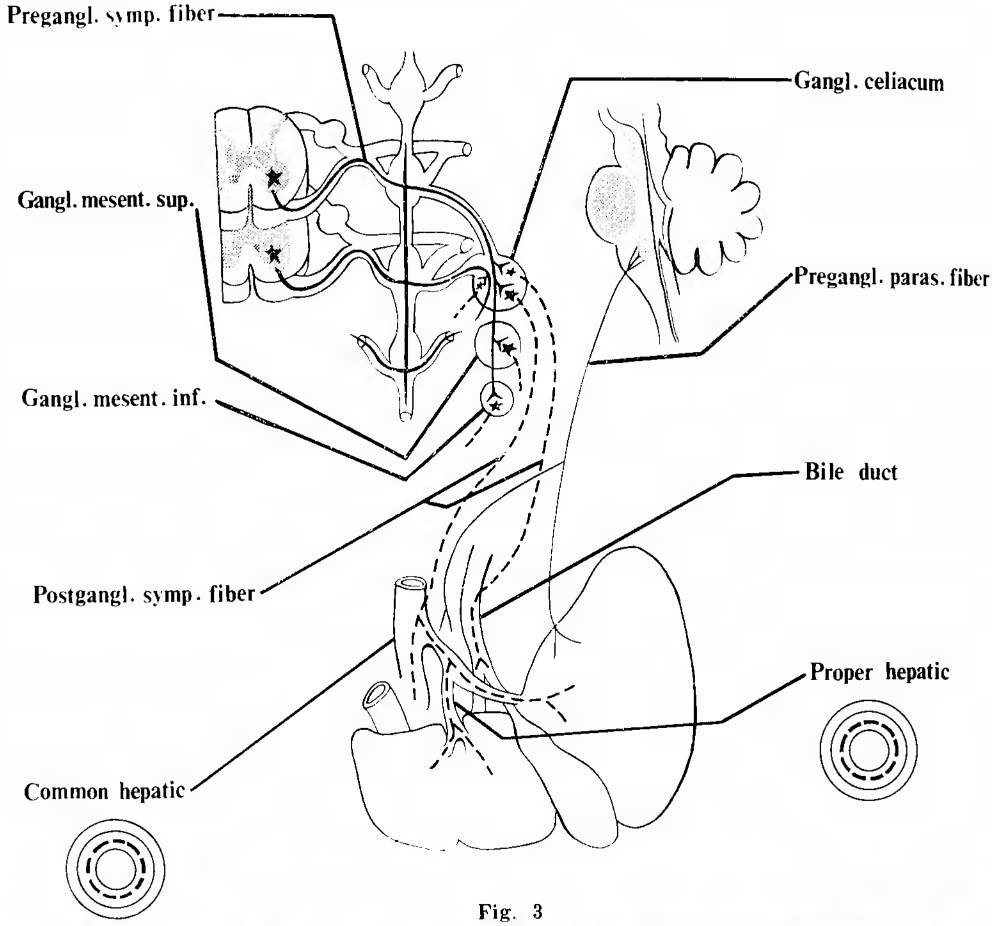


Fig. 3

Fig. 4 Celiac artery of dog.
Cross section.
Specific fluorescence of the adrenergic fibers are found in the smooth muscle layer of the media of the artery. Fluorescence micrograph ×128.

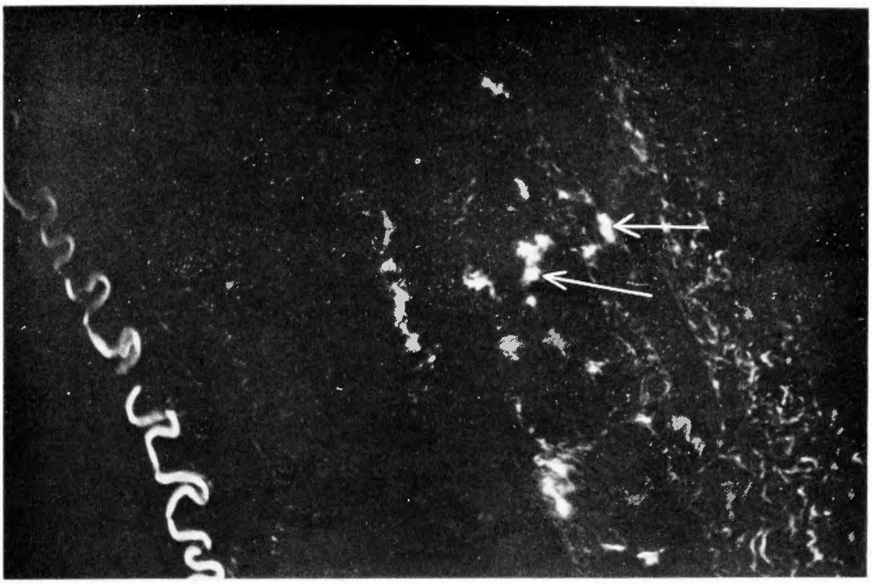


Fig. 5 Hepatic artery of dog.
Cross section.
Several bundles of fluorescent adrenergic fibers are present in the media of the artery. Fluorescence micrograph ×128.

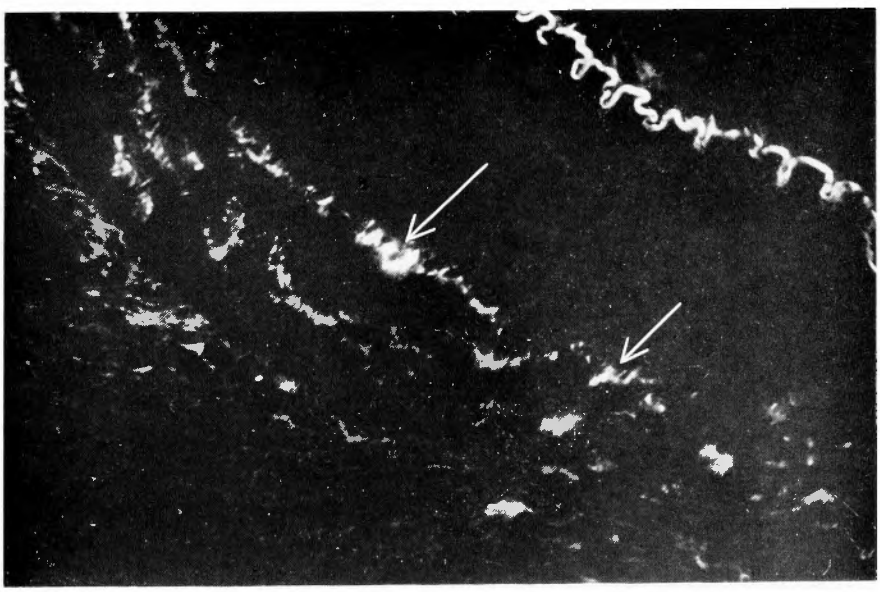


Fig. 6 Proper hepatic artery of dog.
Cross section.
Fluorescent adrenergic fibers are obsecued between the media and the adventitia of the artery. Fluorescence micrograph ×128.

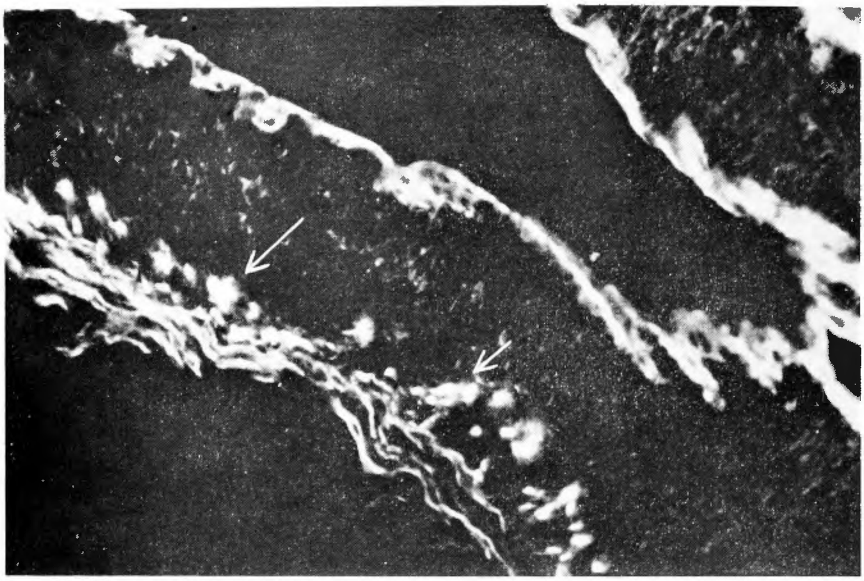


Fig. 7 Gastroepiploic artery of dog.
Cross section.
Several bundles of fluorescent adrenergic fibers are seen, on the leftside, in the media of the artery. Fluorescence micrograph ×256.

Splenic artery of dog (Fig. 8)

Several bundles of fluorescent adrenergic fibers are also present in the media of the artery.

Superior mesenteric artery and its branches (Fig. 9).

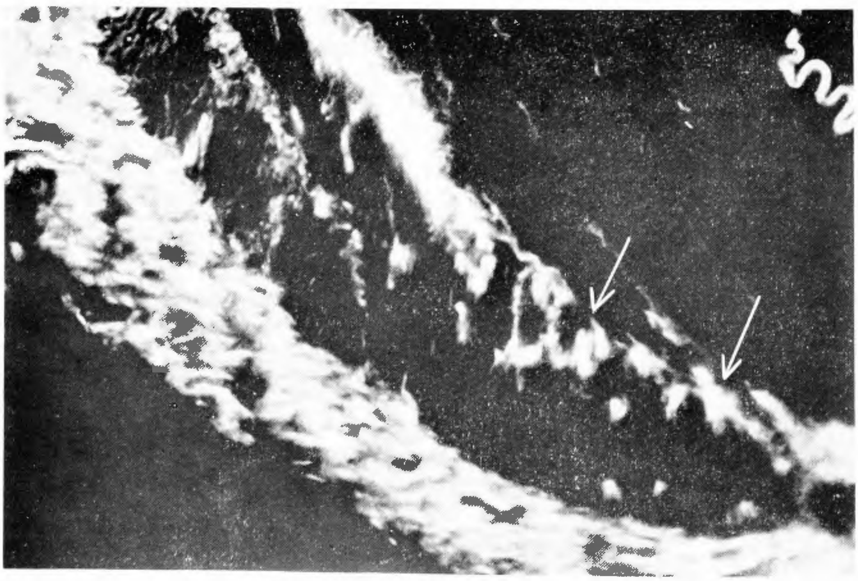


Fig. 8 Splenic artery of dog.
Cross section.
Several bundles of fluorescent adrenergic fibers are present in the media of the artery. Fluorescence micrograph ×128.

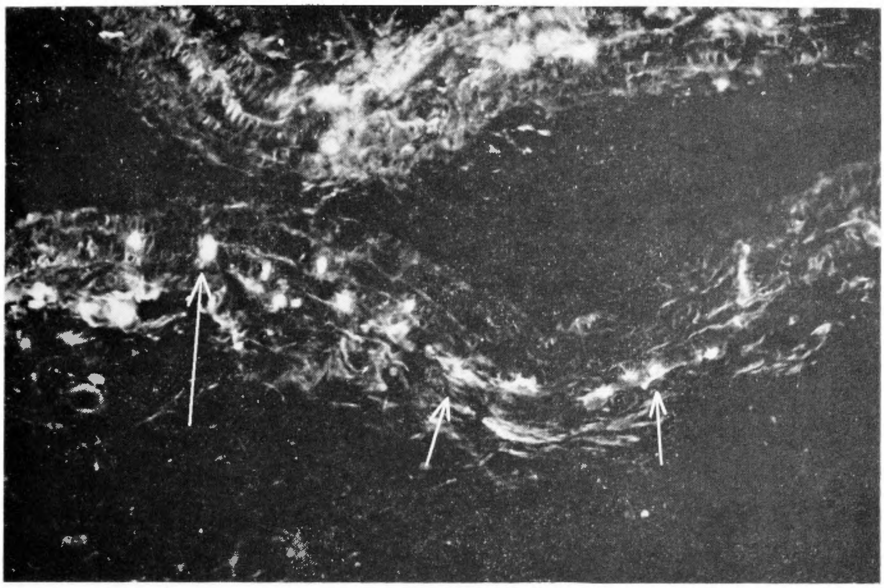


Fig. 9 Artery of mesenteium in human being.
(Branch of superior mesenteric artery)
Many bundles of fluorescent adrenergic fibers are present in the media of the artery.
Fluorescence micrograph ×256.

Fluorescent adrenergic fibers are present in the media of the artery and its branches.

Inferior mesentric artery and its branches. Fluorescent adrenergic fibers are also present in the media of the artery and branches. These fluorescent materials surrounding the arteries were not only directly superimposed on the smooth muscle layer of the media, but also penetrated from adventitia to the smooth muscle layer of the media.

No fluorescence was observed in the abdominal aorta of dog and human.

On the other hand, the fluorescent materials surrounding the abdominal aorta of guine pig were directly superimposed on the smooth muscle layer of the media, but they was seen to penetrate this layer only seldom (Fig. 10). The elastic tissues of the adventitia showed an intense greenish auto-fluorescence, and a bright specific fluorescenct material was found near the border to the media. The specific fluorescence in the media was increased in number and intensity after the injection of noradrenaline. Such an increase lasted for a least 30 min.

Injection of reserpine or chronic resection of sympathetic trunk resulted in a complete disappearance of specific fluorescence in the smooth muscle layer of the media (Fig. 11). Concomitantly, fluorescence of the adventitia also somewhat paled in the greenish tone, as compared with that of untreated animal.

Treatment with sodium borohydride affected the fluorescence of the media and adventitia, in a similar way to reserpine treatment or sympathectomy.

DISCUSSION

Distribution of catecholamine fluorescent adrenergic nerve fibers in the arteries supplying abdominal organs in dog. Two types of fluorescent adrenergic innervation of the arteries were detected by our observation (Fig. 12).

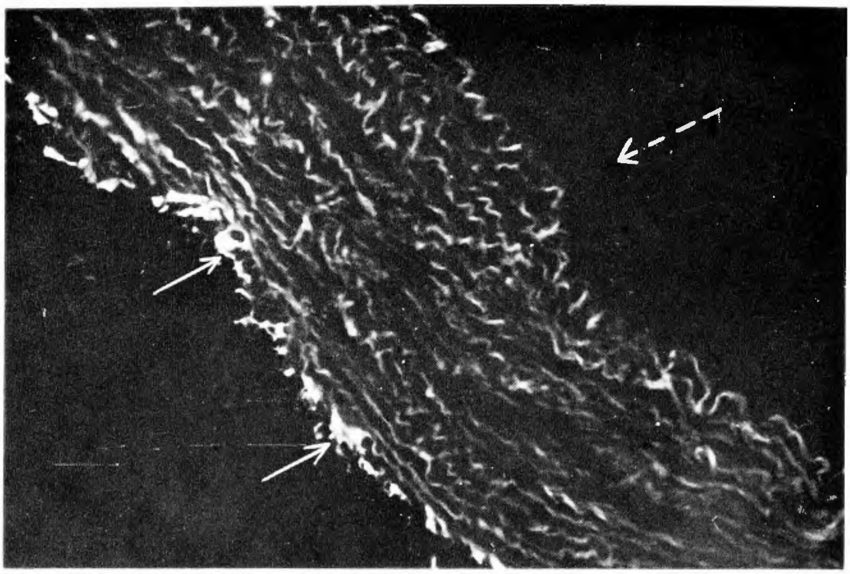


Fig. 10 Abdominal aorta of guine pig
Cross section.
Several bundles of fluorescent adrenergic fibers are directly superimpsed on the smooth muscle layer of the media. Fluorescence micrograph ×128. (→ Intima)

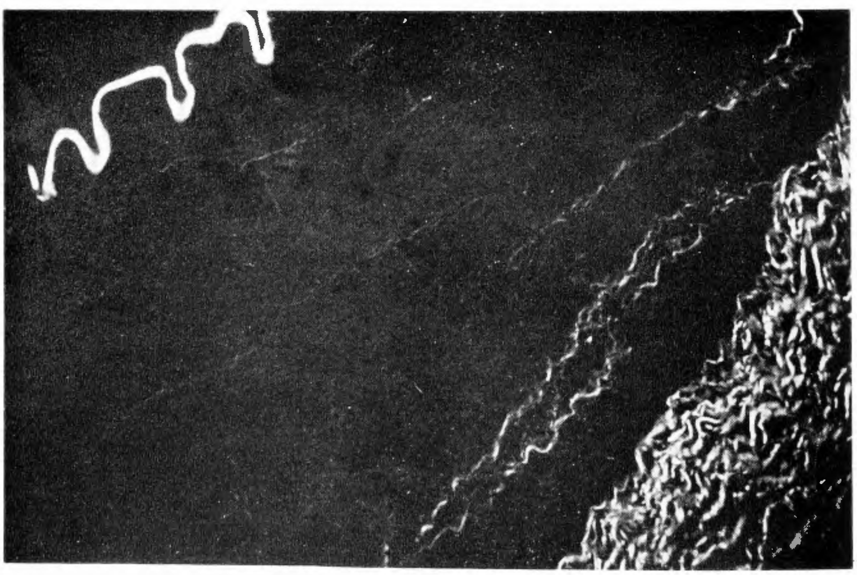
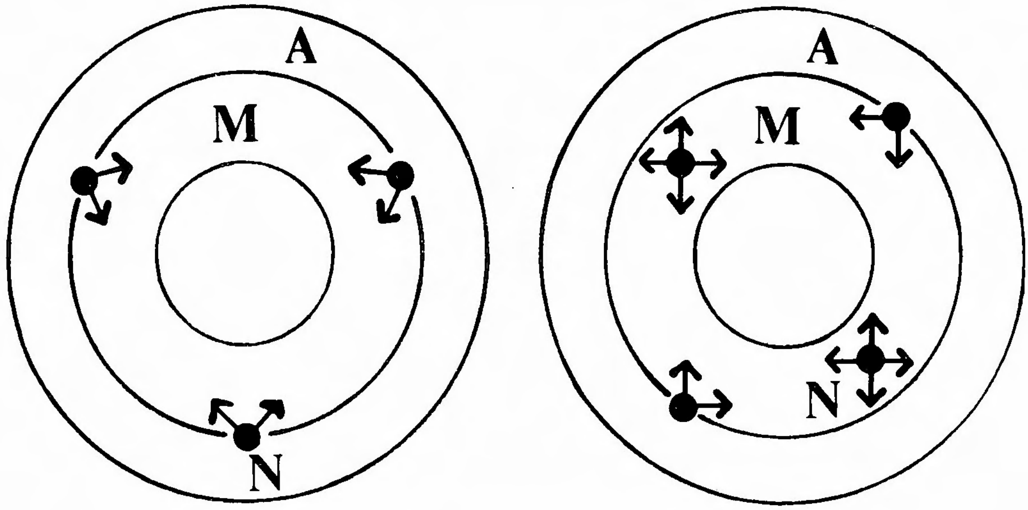


Fig. 11 Celiac artery of dog. Resenpine administration.
Cross section.
No specific fluorescence of the adrenerfic fibers are found in the smooth muscule layer of the media of the artery, after reserpine administration. Fluorescence micrograph ×128.



M : Media A : Adventitia N : Catecholamine fluorescent nerve ending

Fig. 12

Only aorta of guine pig and rat had fluorescent adrenergic nerve fibers between the media and the adventitia, except arterioles. Fluorescnt adrenergic nerve fibers were not observed in aortas of human being and dog with our researches. This type of vessels had fluorescent adrenergic nerves fibers only in the adventitia or between the adventitia and the media, but it had none of them in the media. Other type of vessels such as celiac artery, superior mesenteric artery, inferior mesenteric artery and their branches had fluorescent adrenergic nerve fibers in the media of vessels. Vessels differ in their architectural structure and their behavior according to their varied task, from the stand point of classical histology. There are, in general, four different vessel types. On the arterial side are elastic arteries and muscular arteries, but it is hard to say where the one ends and the other begins, since the structural changes are gradual. Usually the aorta, the subclavian artery, and the common iliac artery are regarded as elastic artery. Arteries more peripheral than the above, down to the arterioles, are classed as muscular arteries. After these are the capillaries. Then there are veins. Therefore it seems that visco-elastic and plastic behavior of elastic arteries depends mostly on elastic tissue, collagen tissue, and ground substance, but only to a small degree on smooth muscles. These elastic arteries with above mentioned physiological properties, such as aorta, had smaller need for sympathetic innervation resulting vasoconstriction. The muscular arteries with the physiological function which controlls the cirulating blood volume, had need for sympathetic innervation to make vasoconstriction.

Silver Impregnation method and Histochemical fluorescence method of catecholamine. With BILSCHOWSKY's silver impregnation method, the vascular efferent fibers are carried in paravascular filaments which can sometimes traced alongside arteries for considerable distances before they break up into finer fascicles. These fascicles unite in an open perivascular plexus from which small groups of fibers penetrate into the vessel walls, sometimes alongside the vasa vasorum, to ramify and form much finer networks in the adventitia

and in the media. Some fibers reach the junctiond and between the media and intima, but it is doubtful if they enter the latter. These appearance led KIMURA, CAJAL and DOGIEL to describe superimposed and inter-conneted networks; the first and best defined in the adventitia a second, very delicate, in the media ; and a third often difficult to distinguisch, in the zone between the media and intima.
But our experimental studies with a recently developed specific histochemical fluorescent method for the detection of catecholamine, showed the above metioned results, including that two types of adrenergic innervation existed in the arteries, an that adrenergic nerve fibers were not yet found in the inner layer of the media and also between the media and intima.

Physiological studies and histochemical fluorescent method for adrenergic nerve.
With physiological studies of many investigators, there is general agreement that splanchnic stimulation increses the resistance to blood flow through the mesenteric circuit. Vagal stimulation probably has little if any significant influence on the mesenteric blood flow, except in sofar as flow is changed secondary to an increase in motility in the stomach and gut. This fact suggests a sympathetic innervation. Therefore this physiological significance was supported by our histochemical findings, which meaned the rich existance of adrenergic innervation on the arteries supplying abdominal organs.

The origin and height of sympathetic innervation to arteries supplying abdominal organs. There was much arguments about origin and heights of sympathetic nerve fibers to the arteries. From the basis of classical anatomy, a large number of pregangilonic fibers does not terminate in the sympathetic trunk but in a group of preaortic ganglia. Fibers from T 5-T 9 fuse to form the greater splanchnic nerve, which terminates mainly in the coeliac ganglion. Fibers from T10-T11 fuse to form the lesser splanchnic nerve, which goes primarily to the superior mesenteric ganglion. Fibers from T12 forms the least splanchnic nerve, which goes mainly to the inferior mesenteric ganglion. The ganglia are located on the surface of the aorta, around the origin of the vessels which give them name. The postganglionic fibers go from here, forming periarterial networks, to innervate the vessels to abdominal organs and also the abdominal viscerae themselves. The exact locarization, height and ramification, from which the sympathetic nerve fibers to the vessels supplying abdominal organs were originated, are now investigated in our laboratory-group, but the above stated opinions were in general accepted by many investigators. Therefore, the elimination of sympathetic tone on the vessels of abdominal organs was, in our animal experiments, performed from the height T3 to L6, but technical difficulties were occured to resect trunk, ganglia and nerves of the heights from last thoracal ganglion to first lumbal ganglion (L1).

From the basis of our studies, regional sympathectomy could cause vasodilatation of splanchnic vasculature. Therefore, sympathectomy is one of useful methods for treatment of intestinal ischemia or arterial occulusive lesions of splanchinc areas, as well as for ischemic change of arterial occulusion in the limbs.

CONCLUSION AND SUMMARY

It is very important that catecholamine-fluorescent nerve fibers are detected to penetrate through the muscle layer of the media in the arteries supplying abdominal organs, as muscular arteries ; celiac artery, gastric arteries, gastroepiploic arteries, splenic artery, gastroduodenal artery, common and proper hepatic arteries, superior mesenteric artery, inferior mesenteric artery and their branches.

Catecholamine-fluorescent nerve fibers are detected very rarely in aorta of some species, such as guine pig or rat, as a elastic artery, between the adventitia and the media of aorta. It is noteworthy, that histological study of artery show two types of arteries, such as elastic artery and muscular artery in their architectural structure and their behavior to their varied task, and also that our histochemical fluorescence method detected two types of arteries with modes of sympathetic innervation, which are described above. These facts might explain the mutual connections and interactions between physiological and histological natures of the arteries.

AKNOWLEDGEMENT

We wish to express our deepest gratitude to Prof. Dr. Chuji Kimura for his helpful advice and kind guidance throughout this study.

NOTE

This work was presented by Dr. K. Mohri at Symposion of International Society for Neurovegetative Research on Neurohormones and Neurohumores, in Amsterdam, July 24, 1967.

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和文抄録

腹部血管のアドレナリン作動性神経の分布に関する研究

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藤原元始・無量林堯

血管に於ける交感神経の発効器は中膜平滑筋であるが，その神経支配については未解決の問題が多く残されている．

本研究は，螢光組織化学的方法により，腹部内臓血管のアドレナリン作動性神経支配について検討を加えた．

筋性血管，又は拮抗血管として総括される腹腔動脈，胃動脈，胃大網動脈，脾動脈，胃十二指腸，総肝及び固有肝動脈，上及び下腸間膜動脈及び，上記動脈の枝にはアドレナリン作動性神経が血管外膜と中膜との境界部に分布すると共に，中膜筋層にまで達している．

弾性血管の1つと考えられている大動脈には，ハムスター，ラットにのみ血管外膜と中膜との間にアドレナリン作動神経を認めるが，犬及び人間には註明出来なかつた．

以上の成績から，弾性血管と筋性血筋の神経支配について，組織化学的証明法により，アドレナリン作動神経分布の差異が認められることは，生理学的実験成績によつても同様に，2種類に分類出来る事実と考え合わせると興味深い．